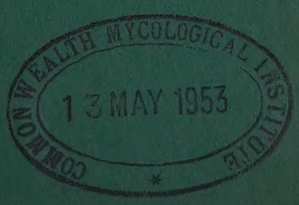
C. Owen: Determination of Available Nutrients in Malayan Soils

THE JOURNAL OF THE RUBBER RESEARCH INSTITUTE OF MALAYA

MARCH 1953

Vol. 14 Communication 282



DETERMINATION OF AVAILABLE NUTRIENTS IN MALAYAN SOILS

By

G. OWEN

Experience has shown that laboratory methods for assessing the nutrient status of soils give results which do not always agree with observations on growth and yield of crops. Methods used for the determination of available potash and phosphate in soils are empirical, designed to give relative rather than actual values. Numerous extracting agents have been proposed but it is evident that no method gives an equally satisfactory evaluation of nutrient status for all soils in all parts of the world. Investigations to determine the most suitable solvents, and the most convenient procedures, for determining available potassium and phosphorus in soils of different countries and regions, have led to the accumulation of an enormous literature which has been reviewed recently in *Soils and Fertilizers* 13 (1950) 235; 14 (1951) 347, but there are few references to work on the determination of available nutrients in tropical soils. MUKHERJEE (1941) recommended Truog's method for determining readily soluble phosphorus in non-calcareous red soils in India; DAMES (1949) mentions that 2% citric acid is used as the extracting agent for soil phosphorus in Java, JONES (1949) prefers 0.5 NaOH to citric acid for Kenya soils, and in Natal, BEATER (1949) found that extraction of soils with N/21 H_2SO_4 containing 0.36 gm of sodium borate per litre gave phosphate values which correlated with those of the 1% citric acid method. BIRCH (1952) concludes that base saturation data may be used for assessing phosphate availability and predicting phosphate response in acid soils of East Africa, but he adds that this may apply only to soils of low or moderate phosphate fixing capacity.

No reference to special procedures for estimating available potash in tropical soils has been noticed and it is assumed that assessment of the potash status in these soils is usually based on values for exchangeable potash as determined by conventional methods.

PROJECT

The methods most commonly used for the determination of readily soluble phosphate in soils are those in which the soil is extracted by dilute acids. The one which has been used hitherto in the Rubber Research Institute of Malaya is the Truog method in which the soil is extracted with 0.002 N H_2SO_4 Although mean values for soils which do not respond to phosphate are higher than those for responding soils, the values are so low and the range so narrow that

it is often difficult to grade individual samples satisfactorily according to their phosphate status. For advisory purposes the method is not completely satisfactory and therefore other methods have been studied and tested in an attempt to determine which of these methods affords the best means of distinguishing between those soils which are deficient in readily available phosphate and those which have adequate supplies for normal development of rubber trees. For this purpose soil samples were obtained from untreated plots in experimental areas where the response to treatment with phosphate is known.

The evaluation of potash status of soils has been based on values for exchangeable potassium determined by extraction with 0.5 N acetic acid. There is nothing to indicate that this method of assessment of the relative contents of available potash is unsatisfactory, but it is to be noted that a response to treatment with potash has been established in only one of many manuring experiments in rubber areas in Malaya, and therefore we have no basis for a proper judgement of the results. It would seem that the content of available potash in the majority of Malayan soils is adequate for normal growth of rubber trees. Nevertheless, the opportunity was taken to compare values obtained for exchangeable potassium with those obtained by biological methods, in an attempt to determine whether a measure of the fraction extracted by dilute acetic acid gives a reliable assessment of the readily available potash in local soils. It was assumed at the outset that values obtained by the biological methods give a true indication of the relative amounts of nutrients available to plants.

EXPERIMENTAL

The two biological methods used as a basis for comparison with the chemical methods are *1* Neubauer method and *2* Aspergillus method.

The Neubauer method is based on the determination of nutrients extracted by seedlings growing over a definite period in a known weight of soil. Rye seedlings are normally used as the indicator plant, but since rye seeds are not readily available locally, rice seedlings were used instead. Preliminary tests were carried out with three strains of rice seeds out of which one was selected (Mayang Sa Batil) and used in all subsequent work. No attempt was made to control temperature as it was thought that diurnal variations in laboratory temperatures here were too small to cause any appreciable effect. Although maximum and minimum temperatures within 24 hours differ by about 20°F, the temperature fluctuation is fairly constant from day to day. The assumption that control of temperature was not necessary may not have been justified since it was found that the results of replicate determinations carried out simultaneously agreed more closely than the results of replicate determinations separated in time. This applies in particular to the phosphate values.

The second biological method is based on the use of Aspergillus niger as a test organism for determination of available nutrients. For the preliminary trials a strain of Aspergillus niger isolated locally was used but later a culture (strain Groningen) from Holland was obtained by the courtesy of Dr F.C. Gerretsen. This proved to be more vigorous than the local strain and was used for the series of tests carried out. The soil sample was placed in the culture solution which was then inoculated with spores of Aspergillus niger and the mycelium was weighed after incubation at 35°C for four days.

The chemical methods used hitherto for assessing the relative potash and phosphate contents have already been mentioned. For phosphate, a second method developed by BRAY and KURTZ (1945) in which fluoride solutions are used as the extracting agents, was tested and the results compared with those obtained by the Truog method and the biological methods.

The soil samples used in this investigation were obtained from control plots in 27 experimental areas on rubber estates in various parts of the country. From each plot several samples were taken to a depth of six inches and these were mixed to form a bulk sample. The bulk samples from each control plot were analysed separately and the number of samples per experiment varied from 2 to 6. Young rubber plants showed a marked response to treatment with phosphate in 21 experiments while in the remaining 6 no response was detected. The effect of fertilisers on growth of rubber plants during the first few years after planting in the majority of these experimental areas have been discussed by AKHURST and OWEN (1950). All experiments in which a beneficial effect to treatment with phosphate has been established are sited on inland residual soils. In the discussion which follows, soil samples taken from these experimental areas are referred to as the responsive soils, while the non-responsive soils are those sampled in the few experimental areas where no beneficial effect due to phosphate manuring has been detected. It happens that all the non-responsive areas are sited on soils derived from coastal alluvial deposits.

AVAILABLE POTASH

The values for readily available potash as determined by all three methods cover a fairly wide range. The range of values obtained are:

	No. of samples	*Range of values*
Exchangeable potash	158	11 to 250 p.p.m. K_2O
Aspergillus values	158	89 to 758 mg (wt. of mycelium)
Neubauer values	76	1 to 20 mg K_2O

The linear relationships of the exchangeable potash values with the Aspergillus values and with the Neubauer determinations are illustrated in *Figures 1* and *2* respectively.

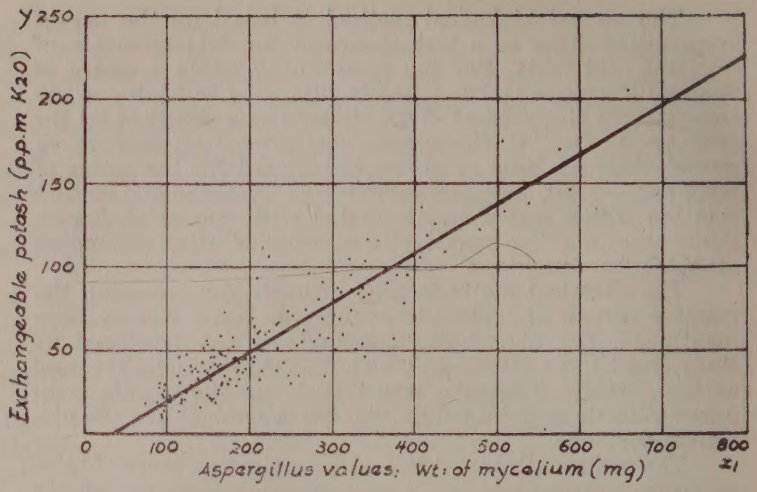


Figure 1. Regression of exchangeable potash values, y, on Aspergillus values, x_1

Regression equation: $y = -9.0 + 0.293\, x_1$
95% *confidence limits of* $y = \pm 31$ *p.p.m.* K_2O
Correlation coefficient: $ryx_1 = +0.92$
Number of samples = 158

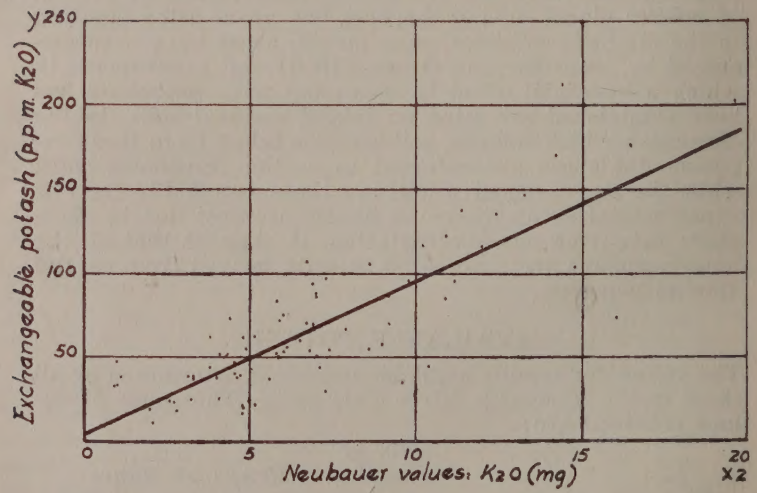


Figure 2. Regression of exchangeable potash values, y, on Neubauer values, x_2

Regression equation: $y = 3.5 + 9.12\, x_2$
95% *confidence limits of* $y = \pm 48$ *p.p.m.* K_2O
Correlation coefficient $ryx_2 = +0.84$
Number of samples = 76

The correlation coefficent relating exchangeable potash values and Aspergillus values, measured over 158 samples, is + 0.92; for exchangeable potash and Neubauer determinations the coefficient, measured over 76 samples is + 0.84.

Both these coefficients represent highly significant correlation and demonstrate the agreement between the results obtained with the three methods. However, the exchangeable potash values appear to relate more closely to the Aspergillus values than to the Neubauer values, as demonstrated by the more significant correlation coefficient and the smaller scatter about the regression line of *Figure 1*.

The Neubauer method has the outstanding advantage that the plants themselves are used as the extracting agent, but it is laborious and it is evident that careful attention to details such as control of temperature and moisture is required to obtain reproducible results. As a routine laboratory method for assessing the potash status of soils it has no special merit, but it can be of value for investigational work and on one or two occasions it has proved useful for diagnosing nutrient deficiency when simpler and more rapid methods failed.

The Aspergillus method also has the advantage in that the assessment of nutrient status is based on growth of the fungus rather than on extraction by a chemical reagent. Compared with the Neubauer method, it is much simpler and less time consuming. Differences between high and low test values can be readily seen merely by comparing the growth of the mycelium, and the method provides a simple means of obtaining a rough assessment of the relative potash status of soils when facilities for chemical analysis are not available.

The chemical method, in which the exchangeable potash is extracted by dilute acetic acid, is however more rapid and generally more suitable than either of the biological methods for routine determinations in the laboratory. The chemical method is empirical but since the values obtained correlate so closely with those obtained by the biological methods it is concluded that the fraction extracted by dilute acetic acid is closely related to that portion of the soil potash which is readily available to plants. Therefore it would seem that a determination of the exchangeable potash by the standard procedure gives a reliable assessment of the relative potash supplying power of local soils.

AVAILABLE PHOSPHATE

BIOLOGICAL METHODS

Both biological methods proved unsatisfactory for routine determination of readily available phosphate in local soils. All values obtained by the Neubauer method are low. Only a few exceptional samples gave values greater than unity and negative values are not uncommon. The chief defect of the method, however, lies in the fact that the results are not reproducible under the conditions in which the pro-

cedure was carried out. While agreement between replicates in any one series of determinations carried out at the same time was reasonably good, reproducibility of the results in subsequent determinations was not satisfactory. There was no significant correlation between the Neubauer values and the results obtained by the standard Truog method.

Values obtained by the Aspergillus method are also very low. Correlation between the results obtained by this method and the Truog values is statistically significant. The correlation coefficient relating Truog values and Aspergillus values, measured over 134 samples, is + 0.35 ($P<0.001$). Although the correlation is highly significant, the agreement is not so close as that between the two chemical methods (*c.f.* TABLE I). Growth of the mycelium, however, is generally poor in tests with all soils examined by the Aspergillus method, and although the mean value for non-responding soils is higher than that for the responding soils, the range is too narrow to allow for a clear discrimination when individual samples are examined. No more accurate estimate of the relative phosphate contents is possible than that which can be obtained by the simpler and more rapid chemical methods.

It is concluded therefore that the biological methods have no special merit and are not recommended for routine assessment of the relative phosphate status of local soils.

CHEMICAL METHODS

Values for the readily available phosphate fraction obtained by the Truog method are very low, and although it is recognised that phosphate is deficient in the majority of Malayan soils, the fact remains that soils which do not respond to treatment with phosphate have values which are not appreciably higher than those for soils which do show a marked response to treatment. Values usually fall below 10 parts per million, with a few in the range 10 to 20 p.p.m. Mean values for responding and non-responding soils are 5 and 12 p.p.m. respectively. The difference is statistically significant, but the values are so low and the range so narrow that it is often difficult when examining individual samples, to determine whether they should be classified as responding or non-responding soils.

It becomes increasingly evident that methods based on the principle that the phosphate soluble in dilute acids is readily available to plants are not completely satisfactory when applied to acid tropical soils. These methods give a measure of the relative contents of a certain fraction of the soil phosphorus which, admittedly, is easily available but it is a fraction which is very small in proportion to the other forms of phosphate which must be available also. It is generally accepted that the mechanism of phosphate absorption by plants is rather more complex than this principle of solubility in dilute acids implies, and it is certain that soil phosphate other than that extracted by dilute acids can be absorbed by the roots.

In a separate study of the distribution of the various phosphorus fractions in local soils (OWEN 1953), it was found that the acid soluble fraction forms a very small proportion of the total amount present and that by far the largest proportion is present either in the alkali soluble form, or in a form which is insoluble in both acids and alkali. Even when phosphate is added to these soils very little remains in an acid soluble form and most of it is converted into other forms. It is logical to assume therefore that at least part of the alkali soluble fraction must play an important part on the phosphate nutrition of plants.

One of the forms of phosphorus extracted by treatment with sodium hydroxide is the adsorbed phosphate. If it is accepted that anion exchange reactions in soils are important, we may assume that adsorbed phosphate is a fraction which plays a part in the phosphate nutrition of plants. It would seem therefore that a determination of both adsorbed and acid soluble forms of phosphate should give a better assessment of the available phosphate status of soils than a determination of the acid soluble fraction alone.

BRAY and DICKMAN (1942) used a solution of ammonium fluoride for extracting soil phosphate and they consider that this reagent extracts the water soluble and easily replaceable fractions. Using different extraction methods with the fluoride solution they attempted to separate the adsorbed phosphate into three fractions. They found that growth and response, for the crops and soils which they studied, generally correlated with the relative amounts of all three fractions, not with the amount in any one fraction.

Later BRAY and KURTZ (1945) proposed a more specific definition of available forms of phosphate and recognise a general division into the adsorbed forms and acid soluble forms. They developed rapid methods for practical use in determining proportional parts of the two fractions, and laboratory methods for the quantitative estimation of each group separately. They claim that the sum of the adsorbed and acid soluble forms ' is the measure of phosphate effectiveness.'

The rapid methods described by Bray and Kurtz were used to determine proportional parts of the adsorbed and acid soluble phosphate fractions in the soils from experimental areas.

COMPARISON OF RESULTS OF CHEMICAL METHODS

The relationships between the Truog values and those for the three fractions measured by the Bray and Kurtz method are summarised in TABLE I.

It will be seen that results obtained by the fluoride extraction method are highly correlated with those of the Truog method. There is little to choose between the separate fractions measured by extraction with fluoride solutions, although the sum of the acid soluble and adsorbed fractions may possibly agree rather more closely with the Truog va-

lues than either of the two fractions separately. The sum of the acid soluble and adsorbed fraction is regarded as the measure of the relative phosphate effectiveness in soils, and it is this value which will be considered further when examining the results from the standpoint of discriminating between responsive and non-responsive soils.

TABLE I

COMPARISON OF TRUOG VALUES WITH VALUES OBTAINED BY THE FLUORIDE EXTRACTION METHOD

No. of pairs of determinations = 154

Truog values (y) compared with:	*Regression equation*	*95% confidence limits about regression line*	*Correlation coefficient*
x_1 *Acid soluble fraction*	$y = 3.73 + 0.545\ x_1$	± 5.68	+ 0.76 ($P<0.001$)
x_2 *Adsorbed fraction*	$y = 2.58 + 0.464\ x_2$	± 5.10	+ 0.81 ($P<0.001$)
x_3 *Total available P* ($x_1 + x_2$)	$y = 2.79 + 0.274\ x_3$	± 4.97	+ 0.82 ($P<0.001$)

TABLE II

ANALYSES OF VARIANCE FOR RESPONSIVE AND NON-RESPONSIVE GROUPS OF SOILS

Soil group	*d.f.*	*Mean Squares*	
		Truog method	*Fluoride extn. method*
Responsive group of soils			
Between experiments	26	25.69 ***	165.52 ***
Between samples within experiments	99	6.01	16.39
Non-responsive group of soils			
Between experiments	5	120.16 ***	1,039.33 ***
Between samples within experiments	22	10.84	109.74

TABLE III

COMPARISON OF MEAN VALUES (P.P.M.) FOR RESPONSIVE AND NON-RESPONSIVE GROUPS OF SOILS

Soils	*Truog method*	*Fluoride extraction method*
Responsive soils	5.30 (± 0.45)	8.98 (± 1.15)
Non-responsive soils	11.75 (± 2.07)	33.46 (± 6.09)

In TABLES II and III the analytical data used as a basis for comparison of the two methods are summarised. It

is evident that both methods make it possible to distinguish between responding and non-responding groups of soils; differences between mean values in each case are highly significant. Applying the Fisher-Behrens test we find that with the Truog method the difference between the means is significant and corresponds to a probability less than 0.05. However, the mean difference between values obtained for responsive and non-responsive soils by the Bray and Kurtz method is nearly four times greater than that for the Truog method, whereas the ratio of their respective standard errors is less than 3, and the corresponding probability is less than 0.01. Therefore, the difference between the phosphate levels of the two groups of soils can be asserted with greater confidence with the former than with the latter method. Furthermore the values for available phosphate obtained by the fluoride extraction method fall over a much wider range than the Truog values, and thus allow for a better discrimination between individual samples.

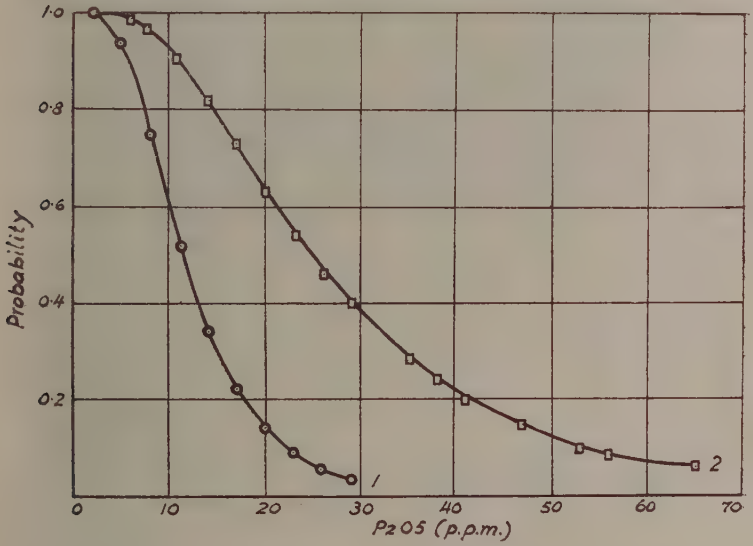


Figure 3. Curves showing probability of response to phosphate manuring based on values for available phosphate as determined by: Truog method (Curve 1) and fluoride extraction method (Curve 2)

It is concluded that where the problem is to differentiate between mean results for a large number of samples, both methods make it possible to distinguish between responding and non-responding groups of soils. But where the problem is to assess the relative phosphate status of individual samples, the fluoride extraction method is more discriminating, and consequently more reliable than the Truog method.

On the basis of results obtained for the group of soils under examination, and using probit analysis technique, it is possible to estimate a response probability curve for each set of values. For example, curve *1* in *Figure 3* shows the probability of response to phosphate manuring corresponding to values on the Truog scale. The so called 'threshold value' or that value of available phosphate for which there is an equal chance of responding or not responding is estimated to be 11.4 p.p.m.; the 95% fiducial limits of this estimate are 9.2 and 14.0 p.p.m. Similarly, curve *2* illustrates the case for the fluoride extraction values. The estimate of the available phosphate on this scale corresponding to a fifty/fifty chance of response is 24.6 p.p.m. with 95% fiducial limits of 20.0 to 30.3.

The fluoride extraction method is simple, rapid and suitable for routine determinations in the laboratory. It is suggested that this method, which measures a proportion of both acid soluble and adsorbed forms of phosphate, is more suitable for the purpose of assessing the relative phosphorus supplying ability of local soils than other methods in which only the acid soluble forms are extracted. The values obtained allow for grading the soils more satisfactorily according to their phosphate status. The estimated value for available phosphate, below which a response to treatment with phosphate can be expected, will vary for different crops. In rubber areas there is a good chance of a response to phosphate manuring when the value for available phosphate in the soil, as determined by the fluoride extraction method, falls below 25 parts per million.

SUMMARY

A comparison has been made of different methods of estimating available potash and phosphate in Malayan soils. For this purpose samples were taken from untreated control plots in a number of experimental areas on rubber estates.

For the assessment of the potash status the methods which were compared are:

1 Determination of exchangeable potash by extraction with dilute acetic acid
2 Aspergillus method
3 Neubauer method

The correlation coefficients relating exchangeable potash values with Aspergillus values and with Neubauer values are highly significant.

No proper judgement based on results from field experiments was possible since a response to treatment with potash has been detected in only one of the several experiments from which samples were taken. But since the exchangeable potash values correlate so closely with the values obtained by the two biological methods, it is assumed that a measure of the exchangeable potash gives a reliable assessment of the relative potash levels of local soils.

For estimating the relative contents of available phosphate in soils results obtained by the two biological methods and by the chemical method proposed by BRAY and KURTZ, (1945) in which the phosphate is extracted by 0.1 N hydrochloric acid and 0.03 N ammonium fluoride, are compared with the results obtained by the Truog method.

Both biological methods proved unsatisfactory. Values are exceedingly low and fall within a narrow range. There was no significant correlation between the Neubauer values and the Truog values. The correlation coefficient relating Truog values and Aspergillus values is statistically significant. Growth of the mycelium, however, is poor in tests with all soils examined and the range of values is too narrow to allow for a clear discrimination between soils which respond, and those which do not respond to treatment with phosphate.

Results obtained by the fluoride extraction method are highly correlated with those of the Truog method. The former values, however, fall over a much wider range and thus allow for a better discrimination between responsive and non-responsive soils. Both methods make it possible to distinguish between responding and non-responding groups of soils when a large number of samples of each group are examined. But in general advisory work the problem is to assess the relative phosphate status of individual samples, and for this purpose the fluoride extraction method is more discriminating, and consequently more reliable than the Truog method.

The probability of response to phosphate manuring corresponding to values for available phosphate as determined by the fluoride extraction method has been estimated. There is a good chance that young rubber trees will respond to treatment with phosphate when the value falls below 25 p.p.m.

The author is indebted to Mr D. R. Westgarth for the statistical interpretation of the data. Thanks are due also to the laboratory assistants in the Soils Division who carried out the analytical work under the author's direction.

Soils Division
Rubber Research Institute of Malaya
Kuala Lumpur December 1952

REFERENCES

AKHURST, C.G. and OWEN, G. (1950) Communication 267 *J. Rubb. Res. Inst. Malaya 12,* 167

BEATER, B.E. (1949) *Plant and Soil 1,* 215

BIRCH, H.F. (1952) *J. agrc. Sci. 42,* 276

BRAY, R.H. and DICKMAN, S.R. (1942) *Proc. Soil Sci. Soc. Amer.* (for 1941) *6,* 312

BRAY, R.H. and KURTZ, L.T. (1945) *Soil Sci. 59*, 39
DAMES, T.W.G. (1949) *Tech. Commun. Bur. Soil Sci., Harpenden 46*, 115
JONES, G.H.G. (1949) *Tech. Commun. Bur. Soil Sci., Harpenden 46*, 180
MUKHERJEE, M.K. (1941) *Indian J. agric. Sci. 11*, 243
OWEN, G. (1953) Communication 283 *J. Rubb. Res. Inst. Malaya 14*, 121

Edited by C. E. T. Mann on behalf of the Board of the Rubber Research Institute of Malaya and printed by The Economy Printers, Kuala Lumpur.